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RESIN SECRETION IN BALSAMORRHIZA SAGITTATA

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(WITH PLATES XXVIII–XXXI AND TWO FIGURES)

Introduction

This problem was undertaken to determine the origin of the secretory tissues and the cause of resin secretion in *Balsamorrhiza sagittata*. The problem was suggested by Professor JOSEPH E. KIRKWOOD, of the State University of Montana, to whom the writer desires to express hearty thanks for valuable suggestions during the progress of the study.

Among the earliest students of secretory organs and their function was MEYEN (13), who stated that "these secretion organs arise from enlarged intercellular passages. One cannot consider them as mere containers, in which the secretion is laid by, but one must compare the containers with their contents to inner glands, and the surrounding walls as specialized glands." This writer proposed that the excretory cells surrounding the secretory canals prepare the balsam and then secrete it through the wall into the intercellular lumen. That the process is surrounded by a sort of mystic vagueness for MEYEN is evident from the description "wonderful" which he applied to the process. In his work on the pine MEYEN (14) found resin not only within the secretory passages and the surrounding cells but throughout the entire stem.

The opinions of the earliest investigators on resin formation were extremely diversified. KARSTEN, WIGAND, WIESNER, and

others of their school considered resin as a destructive slime formation secreted by the cellulose wall lining the cavity, or else a starch derivative. KARSTEN (8) was assured of the intimate relation between the wall and resin gum in the wall, because of the obscurity of the cells in ordinary mounts, whereas the walls became extremely clear when treated with alcohol or ether (p. 317). WIGAND (26) considered resins to be entirely out of the category of secretions, for "a secretion in our sense is only conceivable as a homogeneous material permeable to the cell wall." WIESNER (24) believed the resin masses to be a complex of resin, cellulose, granulose, tannic acid, and "carbonated alkalies," with the cellulose and granulose as intermediate products.

MUELLER (15) and VAN TIEGHEM (22) were unable to find resin in the secretory passages, believing them to be only intercellular spaces. MUELLER was probably the first to use alkannin tincture on dried tissues to test for resin (p. 390). MAYR (12) thought that resin might be secreted by the cells during rapid growth.

Undoubtedly the most careful and authoritative contemporary investigator of resin and the problem of its secretion is TSCHIRCH (21), who has given us a summation of the physiologico-chemical literature of the problem, and in addition valuable evidence contributed from his own studies. TSCHIRCH'S investigations have convinced him that resins and ethereal oils cannot diffuse through membranes which are water-permeable or water-absorbent. All such secretions, he asserts, remain where they were first laid down.

Ecological aspects

B. sagittata was first described by NUTTALL (16) in 1841. The plant is a very conspicuous feature of the landscape of the prairies and south hill slopes of Wyoming, western Montana, and British Columbia. Its leaves are large, auriculate, densely hairy, growing up from the permanent rootstock in April at 3500 ft. level in western Montana. The flower stocks are plentiful. The flowers are golden yellow with conspicuous heads. They begin to bloom about the middle of May and continue until July, although they reach their maximum bloom during June. Very soon after fertilization the flower parts wither, and by the time the seeds are mature in

late July the flower stocks and heads are brown and dry. The leaves remain green until the first heavy frost, when they soon assume a crackling dryness. The plant is a xerophyte, and is commonly found on the flats and upland plains, being especially abundant on the exposed south slopes of the hills. The writer has observed it frequently as high as 6000 ft. and occasionally in the subalpine areas of a still higher altitude.

Specimens of primary rootstock of graduated diameters were dug and dry cleaned and then weighed. They were re-weighed until constant air dry values had been secured. Tables I and II show the results.

TABLE I

SHOWING WATER CONTENT OF PRIMARY ROOTS COLLECTED JULY 1915; COLLECTION DESIGNATED SERIES I

Specimen	1	2	3	4	5	6
Diameter in mm	2.5	4.0	6.0	11.5	13.0	22.0
Weight in gm	0.221	1.429	1.250	2.873	4.195	6.120
Air dry weight	0.088	0.648	0.585	1.505	2.079	3.002
Percentage loss	60.69	54.65	51.45	47.60	50.44	50.95

Average loss of series, 52.63 per cent.

TABLE II

SHOWING WATER CONTENT OF PRIMARY ROOTS COLLECTED OCTOBER 1915; COLLECTION DESIGNATED SERIES II

Specimen	1	2	3	4	5
Diameter in mm	6.0	8.0	9.0	12.0	15.0
Weight in gm	2.337	2.762	3.444	4.144	6.425
Air dry weight	1.107	1.310	1.505	2.038	3.177
Percentage loss	52.60	52.60	56.30	50.80	55.70

Average loss of series, 53.60 per cent.

Tables I and II show a more uniform correspondence for water content in October than in July, although the average water content is practically the same in both series. In general one may conclude that the size of the root has no definite relation to its water content. Within the slight fluctuation the water content is directly proportional to the weight of the root. Also, the average water content is the same at these different seasons of the year.

In direct contrast to these data is the record for water content in random soil samples taken from field areas where *B. sagittata* was growing in abundance. Table III shows such sample records, with normal and air dry weights.

TABLE III

SHOWING PERCENTAGE OF WATER IN RANDOM SAMPLES OF SOIL IN WHICH *B. sagittata* GROWS

Sample	1	2	3	4	5
Weight in gm.....	94.00	126.42	89.27	104.26	110.02
Air dry weight.....	79.11	103.64	77.55	99.12	90.03
Percentage loss.....	15.85	18.02	15.37	4.89	18.18

Average loss of series, 14.46 per cent.

Table III shows a fluctuation of water content in the soil entirely incommensurate with the constant water values of the rootstocks. This may be accounted for in part by the size of the soil particles, since they, too, are far from uniform, and such differences would cause both a difference in weight of soil per unit mass and a consequent difference in capillarity. The fact remains, however, that the plant, irrespective of its root size, selects a relatively constant amount of water from soils that differ noticeably in water content.

Calculations were made also to determine the percentage of resin in air dry roots and leaves. The parts selected were first weighed, then placed in pure ether in an air-tight compartment. They were left in this container for a week, during which time they were shaken frequently. This method of extraction was used after it had been ascertained that ether was the best solvent for the resin of this plant. At the end of this time the ether extract was poured off, filtered, and the ether allowed to evaporate at 20° C. until a constant weight had been secured. For roots dug in July the percentage of pure resin amounted to 3.3; for roots dug in October the percentage was 3.3; for roots dug in May, some three or four weeks after the new growth had begun, the percentage was 5.2. This shows a constant resin value during the resting period and an increased resin content for the growing period. The percentage of resin found in the leaves was 9.8. This value was found for leaves

selected and dried in the middle of May, the time of maximum growth. This resin value was found after the ethereal oil had evaporated. By the osmic acid test it was found to contain no fatty oils. An analysis of *B. terebinthacea* made by Miss HERMA T. KELLEY (**19**) indicated 9.76 per cent resin, 8.96 per cent of which was removed by chloroform and 0.80 per cent by alcohol. In addition to this there were 5.70 per cent oils, 0.42 per cent volatile oils, and 5.28 per cent of fixed oils. LLOYD (**11**) has calculated the percentage of resin for *Parthenium argentatum*, the guayule of the Mexican desert. His values are as follows:

	Per cent resin
Stump	2.46
Wood growth of 1907	1.36
Cortex of above wood	4.06
Growth of 1908	7.56
New growth of 1909 with leaves	2.70
Roots	10.80

These values were obtained from irrigated plants. WHITTELSEY (**25**) secured from 10 to 17 per cent of resin for the field plants of the same species. If the field records are taken, it is evident that by weight the resin content of *B. sagittata* is smaller than that of the related species, *B. terebinthacea*, or of *Parthenium argentatum*.

Associated with *B. sagittata* in a parasitic way is a certain fly of the Typetenid group of the family Muscidae. A complete description of this fly will appear in a separate paper now in preparation by the author. The fly is found in the receptacle of the maturing flower head, living there during the grub and pupal stages of its development. The grub is about 1.8 mm. in length by 0.15 mm. diameter, while the pupa averages 1.5 by 0.15 mm. Usually there is only one individual to the receptacle, but certain receptacles have been observed by the writer in which 5 or 6 of the parasites lived. The grub is very insidious, ordinarily boring a labyrinthine course through the upper parts of the receptacle and into the bases of the maturing seeds. The result is a twofold injury to the seed: an actual destruction of the maturing seed and a stunting of growth in the seed by intercepting the course of nutrition in the receptacle.

Two other important parasites on *B. sagittata* are a nematode and an acarinid. The former is found in the young stem bud before it appears above the ground. The worm eats its way through the bud, mostly in epidermal and cortical tissues, leaving a dry decay behind. Undoubtedly this does much to sap the vitality of the developing vegetative parts, if not entirely forestalling growth. The mite is found in the sinuses between the leaves, sucking out the juices at the bases of the new leaves. Several hundred were found at times in a single leaf bud. This parasite, too, undoubtedly causes serious damage to the plant and serves to control its abundance.

Collection and preservation of material

The material on which this study is based was collected from July to November 1915 and from April to June 1916. Certain roots, stems, and leaf buds were examined fresh, just after collection. Freehand sections were made and observations taken from water mounts. Other material was allowed to dry and was examined as such. However, the greatest part of the material was fixed in various fluids and preserved in alcohol for more detailed examination. Of this last group, material fixed in acid alcohol and preserved in 70 per cent alcohol gave the most satisfactory results. Certain seedlings germinated in the laboratory, illustrating ontogenetic growth, were fixed in Carnoy's fluid. In addition to freehand sections of the alcoholic material, sections of typical roots were made 12 μ thick in series and similar series of the stem and peduncle 8 μ thick. Sections of seedlings were cut 8 μ thick.

Various stains were tried, but the most satisfactory combination was acid fuchsin with malachite green counterstain. This combination gave an excellent contrast, since the lignified hadrome and sclerome elements, as well as suberized walls of the Casparian strip, took on a copper green against the fuchsin background. The ordinary resin stains, cupric acetate and alkannin tincture, were made use of throughout the study. The alkannin was found extremely satisfactory, since it was both specific and rapid. Osmic acid fumes (osmic anhydride) were used to test for fats. Iodine in potassium iodide was employed for starch testing. Chloriodide of zinc was

used to determine the character of the Casparian strip. Slow alcoholic penetration into inulin-testing areas caused a precipitate of this polysaccharide in the shape of sphero-crystals and rhombospheres, while a more rapid penetration caused the material to be precipitated in granular and amorphous masses. Resene was tested for *in situ* by the Mach and Salkowsky-Hesse cholesterol methods (somewhat modified to suit the immediate needs). Crystals of resene found in certain cells were positive to these tests. Similar crystals were found as a check in steam-distilled resene, dissolved in alcohol, and allowed to crystallize as the alcohol evaporated. A more complete discussion of these tests will be found under tests for resene.

The probability of error in resin tests is due in general not to a lack of a specific reagent, but to errors in location of the substance. Due to its solubility in high grades of alcohol it is not impossible that it might become translocated by alcoholic diffusion. Due to its viscous nature it might readily be dislocated in cutting sections from fresh or alcoholic material. The data of certain investigators, among whom are MUELLER (**15**) and VAN TIEGHEM (**22**), show no resin in the resin canals, while SANIO (**18**) and TSCHIRCH (**21**) were unable to find the secretion outside of the canals. Errors in technique must have been responsible for this. TSCHIRCH considered ordinary methods of technique inadequate for the elimination of the error and made use of a method adapted from MUELLER (*loc. cit.* p. 390). He dried the material at 100° C. for some time before cutting. He then stained with alkannin tincture in water (2 parts of the tincture and 5 parts of water). The former procedure allowed all volatile oils to be driven off and hardened the resin to a tough gummy consistency, so that it was not easily removed from its original position by the section cutter. The latter diluted the tincture so that the resin would not readily dissolve in the alcohol. By this method TSCHIRCH was able to demonstrate resin in the form of a dense slime in the canals of *Imperatoria Ostruthium*, *Arnica montana*, and in the leaves of *Abies pectinata* and *A. Normanni;* while the surrounding tissue, especially the secretory cells, was free from resin content. The writer has given due weight to this possible source of error, and has made many preparations from

live material, alcoholic, and dried preparations. It is only by a study of all these preparations that he feels able to present authoritative data.

Germination tests

The seeds of *B. sagittata* are ripe about the first week of July. From that time they soon become dislodged from the receptacle and fall to the ground. Between July 6 and July 15, 1915, several thousand seeds were collected and sorted into two tentative groups, those considered viable and those considered non-viable. The latter group comprised about 90 per cent of the whole. Of this non-viable group almost half were eaten at the base of the seed by the Typetenid parasite, and the remainder were small and shriveled, due to lack of nourishment. This non-viable group was discarded. Of the seeds saved, 100 choice ones were selected October 19, 1915, and weighed. Their total net weight was 1.041 gm. They were then soaked in concentrated sulphuric acid for 8 minutes, carefully rinsed in distilled water several times, and placed in a sterile moist chamber at about 30° C. during the test. The record is as follows:

SERIES I

October 19; 100 selected seeds weighed, sterilized, and set to germinate in sterile moist chamber.

November 3; one seed beginning to burst testa; hypocotyl protruding.

November 5; 3 seeds burst testa; hypocotyl of one 11 mm. long.

November 6; 13 seeds found soft and decaying; thrown out.

November 10; 11 seeds found soft and discarded.

November 11; 5 seeds germinating; 4 thrown off testa.

November 12; 12 seeds found soft and thrown out.

November 14; 8 seeds germinating.

November 17; mold developing; those seeds not yet germinating but considered sound rinsed in weak formalin solution, then thoroughly rinsed in distilled water.

November 28; 10 seeds germinating; 5 of these fixed in Carnoy's fluid, 5 transferred to cork supports in beakers of water and allowed to continue growth; all ungerminated seeds discarded.

Later, no further growth.

SERIES II

November 18; 100 seeds selected, soaked in sulphuric acid for 5 minutes, thoroughly rinsed, and set to germinate between damp filter paper in chamber as in Series I; average temperature 30° C.

November 28; mold developing; seeds rinsed in formalin solution, rinsed in distilled water, and returned to damp chamber.

December 1; culture found dry; had been dry about two hours.

No germination in this series.

SERIES III

January 25; 100 seeds selected, soaked in sulphuric acid, thoroughly rinsed in distilled water, then placed in sterile moist chamber between filter paper; distilled water supplied as needed drop by drop by siphon apparatus; temperature 25° C.

January 31; first seed bursts testa; no mold.

February 1; 5 seeds found soft and discarded.

February 10; 3 seeds germinating.

February 18; 4 seeds germinating.

February 24; 6 seeds germinating; no mold.

February 29; 8 seeds germinating; several of the remainder soft, discarded.

March 4; seeds dry for several hours; no subsequent germination.

An examination of these records shows certain interesting and significant points. A comparatively small percentage of seedlings germinated from selected seeds, due to lack of viability in apparently viable seeds and to infection during the germination tests. An extremely small percentage of seeds germinated from the total seed production. Series I gave a total of 10 per cent of seeds germinated from 100 selected seeds. Series II gave no germination, due to desiccation antecedent to expected germination. Series III gave an 8 per cent germination within the same time limit as Series I (less one day), but at a lower average temperature. The average for Series I and III is 9 per cent. A more elaborate and critical study of the germination values for *Parthenium argentatum* by KIRKWOOD (**9**, p. 39) gave 10.8 per cent for selected seeds of that species.

Since the selected seeds comprised only about one-tenth of the total seeds produced, an average of less than 1 per cent (0.9) is obtained for the ratio of seeds germinated to the total of seeds produced. Although the plant is a perennial, the severity of the winters in the exposed places where the plant grows kills out many of the rootstocks. Taking into consideration the infection of the bud and the stem by nematodes and mites, an enormous seed production would seem necessary to maintain the plant as the dominant member of the society in which it grows.

A survey of field plants was made during May 1916. Plots covering areas 300 ft. square were studied, and the number of root-stocks counted and the seedlings in those areas listed. For two such plots about 800 plants were found, equally divided between the two plots. This number comprised all plants of *B. sagittata* of all sizes and ages within the plots. An accurate idea of the distribution of the plants is seen in text fig. 1. Areas 4 ft. in radius were

FIG. 1.—Field of *Balsamorrhiza sagittata* in vicinity of Missoula, Montana, in May 1916.

closely inspected around each plant, the plants receiving numbers as the listing progressed. In plot 1, in the count of the first 100 plants, one seedling each was found for numbers 2, 3, 4, 8, and 100, no other plant having the seedling within this radius. In plot 2, for the first 100 plants counted, numbers 11, 49, 69, and 70 had one seedling each, while number 68 had two. In a second 100 in plot 2, numbers 41, 61, and 91 had one seedling each. Of those plants observed about half had borne seeds the previous year, or

about 200 per plot of 300 square ft. had been seed producers. Yet only 5 seedlings were found in the count in plot 1, only 6 in the first count in plot 2, and only 3 in the second count in plot 2, averaging 4.66 per cent, a much lower average secured than for seeds germinated indoors. It is evident from the dominance of this species in the society in which it lives that it depends largely upon the continued growth from the rootstock from year to year for maintenance of its dominance. It is not unusual for the individual rootstock to produce 100–300 seeds. This would more than replace the plant each year if the laboratory germination test were effective in the field, but the lower germination record for field plants indicated beyond a doubt that the plant could not be replaced each year by the new seedlings.

The germination in the field is comparatively late. The first of the consocies to germinate is the seed of *Lupinus ornatus*, which begins about March 1. Since *B. sagittata* does not fruit until the third or fourth year, but gives up all the time and energy the first two years to growth and food storage, it is evident that early germination is not essential to the best interests of the plant; yet the blooming rootstocks of *B. sagitatta* are in flower long before the lupine.

Of the factors determining germination, air (oxygen) is undoubtedly the most important. A test of this factor was made in a group of seeds not included in the series just cited. The same conditions prevailed in this series as in the recorded series, except that they were covered with a sterile crystallizing dish so as to exclude air. There was no germination. A careful comparison with the recorded series seems to indicate that oxygen is more necessary to prevent fungous growth than as a factor in the metabolic processes of germination per se. When seeds are once set to germinate, moisture is constantly necessary for germination, as indicated in Series II and III.

The temperature coefficient of germination is interesting. It is evident that germination is more rapid at first at 30° than at a lower temperature. However, although germination at 25° is slower, that appears to be a more advantageous condition, since at that point a maximum growth of the plant is effected for a

minimum growth of fungus. Undoubtedly under field conditions the temperature is constantly less than 25° C., except for a short time during the warm afternoons. In fact, practically any night during the germination period (middle of April to middle of May) a freezing temperature may be recorded.

Certain seeds which actually germinated or commenced to germinate had been injured in the region of the root cap or even in the region of the meristem of the root. This was the cause of a decreased vitality in the entire plant and was often the occasion for rapid bacterial infection. This injury was originally due to the Typetenid parasite in the receptacle of the flower head. Such an injury must be a source of constant decay to germinating seeds in wet ground.

Structure

ROOT.—In the developing seedling of *B. sagittata* at a very early stage, a day or so after the seedling begins to break through the testa, certain cells begin to differentiate into protoxylem. These occur at four angles of the root section, forming a tetragon, giving rise to the tetrarch structure of the primary root. At first these spiral tubes develop singly, but may later be followed by one or two others centripetally at each angle of the tetragon (fig. 7). As might be expected from their later origin, these secondary spiral vessels are somewhat larger than the elementary vessels. At this earliest differentiation of protoxylem there are no indications of protophloem from procambium. Very soon, however, such differentiation begins midway and slightly centrifugal to the line joining the first quartet of protoxylem elements (fig. 8). The procambium cells in this region divide tangentially, with apparent irregularity, developing protophloem externally and at the same time intermediate protoxylem internally. Such growth is represented in figs. 9 and 10. These periclinal divisions continue until 4 or 5 concentric rows of phloem are formed and until the xylem almost completely envelops the axial plate. At this time the axial plate is still composed of undifferentiated tissue quite irregular in contour, strikingly similar to the stem pith of the plant. The leptome strands are limited externally by the undulating endodermis, con-

spicuous now (fig. 10) by anticlinal suberization. The appearance of the thickenings is knotlike or looplike along the radial walls. The endodermis, unlike that of *Parthenium argentatum*, contains no starch grains such as commonly occur in higher plants.

The secondary xylem contains not only well defined spiral vessels and tracheids, but vessels of intermediate type. For instance, in fig. 11, *c* and *d* with bifurcating spiral reinforcements are not far removed from *a*, the true spiral type, while *e* more nearly approaches the eyelet type so characteristic of the tracheids.

In the dicotyledons the usual type of axial structure is parenchymatous; but such is not the case in *B. sagittata*, for there the wood elements soon work centripetally, crowding against the original plate cells. The latter become sclerified, so that the plate becomes a solid disk of vessels and sclerome. Such sclerification begins before radial suberization of the endodermis and considerably earlier than resin duct formation. The centripetal crowding with the addition of the new xylem elements increases the actual size of the region within the cambial ring.

The suberized endodermis serves a twofold purpose. The suberization thickens the walls and allows the endodermis to act as a supporting girdle, and, in addition, acts as an impervious barrier against an external translocation of food material. RUSSOW (**17**) has described two types of suberization of endodermis, that in which the radial and one tangential walls are thickened (his "C" type), and that in which the entire wall is thickened on all sides (his "O" type). HABERLANDT (**6**, p. 372) suggests that such distinction is not of great mechanical importance, since variations may occur within the same genus, such as *Carex*, *Smilax*, etc. Although the "C" type is the most usual in *B. sagittata*, there also occurs the "O" type, and in woody secondary roots a thickening which may be designated as an "H" type (fig. 12). In the primary root of 5 mm. or over, the suberized endodermis is interrupted in regions between resin canals by phloem strands which cross into the cortex in these regions, leaving open an avenue for translocation of materials in these special places (fig. 13, *ph*). The origin and development of the resin canals will be discussed later in this paper.

In the older rootstocks of two or more years' growth three regions may be distinguished, a basal primary root, a median swollen region, and two or more branched root growths above the swelling. From the upper reaches of these proximal root branches arise the aerial portions of the plant system. The lowest root region is characterized by a single row of resin canals and an axial stele, while both of the other parts have two concentric rows of resin canals (fig. 14). Cross rays connect these longitudinal canals at frequent intervals. These old rootstocks are further characterized by lysigenous splitting of the now functionless rays, so that the wood is split apart in almost every ray region (fig. 15, l_1, l_2). This cracking is probably caused by tension in the wood areas and a shrinking of the cells in the near vicinity.

The subsidiary root system of *B. sagittata* varies from the main system in that it is diarch in type. The protoxylem first becomes differentiated as two groups at opposite poles, with evidence of protophloem developing intermediately (fig. 16). By the time the suberization of the endodermis occurs, intermediate wood elements have developed and the axial plate is well sclerified (fig. 17). It is not until considerably later that the resin ducts arise (fig. 18).

The root of the plant has a rather large wood area compared with the extra-cambial portion of the root. Table IV shows that it is practically a ratio of two to one through all stages of secondary thickening.

TABLE IV

Number	Diameter of root	Ratio
1.....	2 mm.	2:1
2.....	3.5	2:1
3.....	4.5	2:1
4.....	12	8:5
5.....	12	2:1

This excess of wood tissue may be accounted for by the area occupied by the rays extending between the wood elements. In no. 5, with two rows of resin canals, lysigenous cracks in the ray region occupy about half of the wood area.

While the tracheids conform to the usual type for Compositae and the phloem cells show no unusual characteristics, certain

features of the stone cells deserve special consideration. These cells are found principally in the hypodermal region and give a hardness to the cortex, which makes untreated material difficult to section. They take on a vivid green with the malachite stain. They are somewhat larger than the surrounding cortical parenchyma, due to their thickenings. In surface view they present a polygonal appearance, with bluntly rounded corners (fig. 19, *a–d*). A view at the edge of the cell shows circular pores which enlarge and approach one another as they invade the center of the cell. The center of the cell is an irregular space devoid of the sclerified material, usually filled with ordinary parenchyma cell protoplasm. This content fails to react to starch, oil, or resin tests. As the canals of the cells near the lumen, they anastomose in pairs or triplets, giving an appearance as shown in fig. 19, *d*. The cells have at least one transverse diameter longer than the longitudinal (compare fig. 19, *c* with *d*). This same type of stone cells also occurs in the axial plate of old woody roots (both primary and secondary), and in the wood of subsequent formation, although it is never found in phloem regions. In the latter tissues it is supplanted by bast strands (fig. 13). The stone cells usually occur in groups of five or six.

STEM AND PEDUNCLE.—The hypocotyledonary stem contains the tetrarch arrangement, as shown in fig. 20. The phloem is exarch and the xylem endarch, with protoxylem innermost. As progress is made up the stem, the meristematic region where the bud resides is approached, containing secondary stem, leaf, and flower structure. At this place the four main strands each give off two anastomosing bundle strands to the bud, while the major portion of the bundle strands continues into the cotyledonary collar (fig. 21). Slightly above the section diagrammed in this figure certain changes occur in the bundle strands. These are best illustrated by a comparison of the section shown in fig. 22 with fig. 24, a diagram of the course of the bundles, seen longitudinally. Between levels *cc* and *dd* strands are given off from *w* and *x*, which unite above *dd* to form a median strand *p*. Coincidentally laterals from *y* and *z* form the median strand *s*. Similarly above the section *dd*, *x* and *y*, *z* and *w*, give off subsidiary strands which anastomose in pairs to form respectively

r and *t*. A section taken between *cc* and *dd* might show all the way from 4 to 12 strands, depending entirely upon the exact level of the section, and a section taken above *dd* might show from 6 to 8 for the same reason. Slight variations in the origin of coincident laterals due to unequal nourishment would be shown in an odd number of traces. Returning to figs. 20–23, diagram 22 occurs about the level *dd*. Laterals from *w* and *x* have been given off to form *p*, but have not yet anastomosed. A lateral from *z* to form *s* has been separated from the parent bundle, but its mate from *y* is still intact within *y*. Meanwhile traces from *x* and *z* have already arisen for the formation of *r* and *t*, although their mates are still within the main bundles *w* and *y*. Hence the actual derivations are atypical in location, although the end results are the same, that is, 4 median strands (*p*, *r*, *s*, *t*) derived from uniting limbs of the 4 original bundles (*w*, *x*, *y*, *z*). The section in fig. 23 shows a level above *dd*, where laterals are being derived from *t* and *w*, *y* and *s*, *y* and *r*, to form strands of tertiary rank, with laterals from *w* and *p* not yet derived. Already *x* and *z* have been broken up by a twofold bifurcation.

Certain atypical traces were found in the study of the tissues of *B. sagittata* at this period in its development. In one series of sections the laterals from *x* and *p* received a trace from below. Further observation showed this trace to end blindly at a lower level. In another series the lateral from *z* to *s* was found to give back certain strands to *z* before the lateral united with its mate from *y*. In such cases transverse sections alone would be difficult to use in tracing such bundle anatomy. In older stems and in the peduncle 8–24 traces are derived, dependent on the amount of conduction required in these parts.

LEAF.—The leaf type of a seedling is defined with reference to the number of traces in the blade which appear as separate entities at the origin of the leaf blade from the petiole. In his studies on some 50 seedlings of representative groups of Compositae LEE (10) has chosen *Silphium perfoliatum* as the type for Heliantheae, to which tribe *B. sagittata* belongs. The general superficial appearance of *S. perfoliatum* and the plant under consideration is very similar. Both seedlings are large and hardy, with no secondary

roots up to this period of development. In writing about the bundle strands of this type LEE states as follows:

> As usual in this order the single vascular bundle at the apex of the cotyledon first divides into 3, after which, in correspondence with the large size of the cotyledons, each main strand gives off a large number of smaller bundles. At a lower level, these begin to re-fuse with the larger strands, and at the base of each cotyledon only 5 vascular strands remain, a large median one and two smaller laterals on either side. In the pronounced cotyledonary tube the extreme lateral and smallest bundles fuse with the corresponding bundles from the other cotyledon, and the composite structure produced, after decreasing in size, moves around and joins on to one of the remaining strands. At a still lower level in the cotyledonary tube, the remaining lateral bundles fuse in pairs, so that 4 canal vascular strands enter the hypocotyl.

Upon examination of seedlings of *B. sagittata* it is evident that neither the cotyledons (figs. 22–24) nor the first true leaf (fig. 6) possess bundle traces exactly corresponding to the type for the Heliantheae. There are considerably more than 5 strands for the region above the origin of the blade (fig. 23), but at a level just below the cotyledons in the cotyledonary collar (fig. 24, level *dd*) only 6 strands are found, although in certain sections even below this level (fig. 22) a greater number is indicated, due to peculiarities of transverse anastomoses. Even the true leaf (fig. 6) shows only 3 bundle strands at the origin of the blade from the petiole. It may be said, therefore, that for *B. sagittata* we have a type of bundle anatomy of somewhat fewer strands than for *Silphium perfoliatum*. With these exceptions it has a general resemblance to the tetrarch anatomy of the Heliantheae.

RESINIFEROUS DUCTS.—A root of a young seedling with cotyledons not yet outspread shows clearly the resin secretion from the protoxylem outward through the cortex. There are large drops of resin at the time the endodermis begins to take on suberized thickenings, yet at this stage no resin ducts have formed. Not until the seedling is some 60 days old do the ducts begin to form in the root. The development, although surely determined beforehand, does not occur until after resin formation. The method of development is schizogenous. First a periclinal division occurs in the endodermal cell opposite a group of tracheids. This is followed by an anticlinal division, so that 4 cells arise from the original endodermal cell

(fig. 26). A lumen develops in the midst of the 4 cells, which canal becomes the cavity for resin secretion. Usually the 4 cells now divide obliquely with new planes of division parallel to the walls of the duct, so that the duct becomes lined with 2 layers of cells (figs. 27, 28). A consequent cleavage at right angles to the walls of the duct gives rise to 8 cells immediately lining the duct (figs. 29, 30). This ring of ducts in the cortex, just outside the endodermis, is the usual complement of ducts for the root. As the root grows, however, room is made between the older ducts and new ones are formed. The resin ducts of the root are continuous from the basal region to the junction of the root with the stem. These ducts are somewhat more undulatory than are the tracheids. At times there is evidence of the fusion of 2 ducts, but this is merely due to a breaking down of internal processes from the cells surrounding the lumen rather than an anastomosis.

An examination of seedlings of 2 mm. or over shows in the hypocotyl 2 concentric series of resin canals, the outer series continuous down through the entire root system, and the inner merely potential in the younger seedlings. The 2 series are connected by radial canals between the longitudinal lumina of the series and by transverse canals between consecutive longitudinal canals of the same ring (fig. 31). Moreover, the inner series is capable of ventral extension in roots of one year or over, so that they extend down and around the median enlargement of the root. At this place they all anastomose in a common center (fig. 14).

This type of concentric rings with radial anastomoses corresponds to observations made by CALVERT and BOODLE (2) for *Manihot Glaziovii*, but is the reverse of LLOYD'S (11) observations on *Parthenium argentatum*.

The ducts in the stem consist of 2 separate systems. These systems have similar origin and structure, but different location. One series is found in the pith opposite the wood of the bundles, while the other series occurs in the cortex opposite the interfascicular region, almost within the interstices between the phloem of the bundles (fig. 25). These ducts arise somewhat earlier than those of the root and apparently are not connected with those of the root system in any way. They are continuous throughout the

entire stem, although they are intercepted in certain regions by processes from the lining cells, as shown in fig. 35. The origin of both these systems in the stem is schizogenous and follows the same sequence of development as outlined for the duct system of the root. HOLM (7), working on the anatomy of *Solidago odora* (pp. 252–254), quotes VAN TIEGHEM as saying that resin ducts have only been observed in the cortex (primary) "in certain species of *Solidago*, including *Kleinia;* otherwise these ducts are frequent in the pith and in the secondary tissues." The two series of ducts in the stem of *B. sagittata* indicate a composite type of duct anatomy, in that they supply a duct system in the primary cortex, hitherto observed only in species of *Solidago*, and in addition supply the usual system of the pith. These ducts, too, are subsequent to resin formation in the stem.

The resin ducts of the leaf are merely upward prolongations of the stem systems, corresponding to the bundle trace relationships already indicated. For each bundle in the leaf there are two canals, one occurring on the upper side of the leaf and the other one on the lower side opposite the hadrome elements. DEBARY (4) gave a very complete table of the duct systems as far as they had been worked out in his day, VILLUEMIN (23) has studied it in certain species, and COL (3) has added to the knowledge of the subject, but a thorough revision of the literature needs to be made in order to bring the knowledge up to date.

Since VAN TIEGHEM prepared his schematic outline for types of resin duct distribution in the stems of Compositae, at least two new types have been observed, namely, the *Solidago* type described by HOLM (7) and the type represented by *B. sagittata*, described in this paper. For this reason it is necessary to reconstruct VAN TIEGHEM'S scheme to include the more recent observations.

OUTLINE KEY TO SECRETORY PASSAGES IN STEMS OF COMPOSITAE TYPES

I. Stem containing passages within bundle sheath
  A. Passages confined entirely to medullary region.... *Ageratum conyzoides*
  B. Passages both within and without bundle strands
    1. Only one medullary passage for each leaf trace bundle
      a) One medullary and one cortical passage

i. Both passages opposite the bundle.....*Solidago limonifolia*
ii. Medullary passage opposite the bundle, but cortical passage in the interstices between bundles..*Balsamorrhiza sagittata*
b) One medullary and several cortical passages *Serratula centauroides*
2. A group of medullary passages for each group of cortical ones
a) Group compact.....*Carduus pycnocephalus*
b) Groups in curved series.....*Helianthus tuberosus*

II. Passages wholly without bundle strands
A. Passages external; not walled in on inner side by endodermis or pericycle.....*Solidago odora*
B. Wall of passages partially formed by endodermis or pericycle
1. Passages single, not in groups
a) One passage in middle of outer margin of each main leaf trace.....*Senecio vulgaris*
b) One passage in middle of outer margin of each main leaf trace; in addition one passage for each single bundle in such united trace.....*Aster* sp.
c) One passage on each side close to phloem of each main bundle trace.....*Tagetes patula*
2. Passages in groups
a) Three to five passages opposite outer margin of phloem and of main bundle.....*Silybum marianum*

Physiology of resin secretion

Numerous theories have been proposed to explain the origin of resin and the methods of resin secretion. Among the more important sources conceived as a basis for resin formation may be named the following: starch, cellulose, tannic acid, phloroglucin, a hypothetical glucoside, terpene, and even chlorophyll. As diversified as are these substances, there may be at least superficial reasons for relating resin to any one of them. However, only a deeper analysis of the problem, following out a particular coincidence of resin and one of these materials, will show whether the relationship is a genetic one or not. Evidence is here presented showing certain relationships of the resin secreted by *B. sagittata*.

The resin of this plant appears as a viscous exudation, especially from newly dug roots. It is a light lemon color in smaller quantities, but in larger amounts (ether extraction) it appears a golden yellow. It contains a small amount of essential oil, but gives no

tests for fatty oils. In the roots of young plants (two years or less) it is found mostly in the outer ring of canals, while in old roots it occurs in the two concentric rings of canals, together with the radial anastomoses.

As has previously been mentioned, the ordinary resin tests are cupric acetate and alkannin tincture. The acetate requires several days and imparts a brilliant emerald to the resin. The alkannin causes the resin to take on a brilliant crimson in a very short time. The resin may be distinguished from oils of a fatty nature by the osmic anhydride test. The alkannin is much more soluble in the higher grades of alcohol, but such a high concentration of the solvent is not desirable, since it also acts as a ready solvent for the resin.

The TSCHIRCH test for resin, modified from MUELLER, was used by TSCHIRCH for demonstrating that resin was present in the lumina of canals of *Imperatorium Ostruthium*, *Arnica montana*, *Abies pectinata*, and *A. Normanni*. In fact, TSCHIRCH noted a layer of slime among all schizogenously formed canals. The writer has made use of this technique for testing resin in *B. sagittata* and *Parthenium argentatum*. These preparations show resin in the canals, as described by TSCHIRCH, but in addition demonstrate resin in the newly formed xylem, an abundance of it in rays and inner regions of cortex, including the cells immediately surrounding the canals, and *great masses of resin in the cambium*. Such dry preparations demonstrate resin in the identical locations as the aqueous mounts from fresh material and alcoholic material. In this wise an accurate check has been secured on the demonstration mounts.

An analysis was then made to discover the approximate relation of resin to other organic materials. Resins are classified according to their reactions to four kinds of tests: resino-tannol, resene, resiniferous oil, and resinic acid tests.

The resino-tannols are those resiniferous materials which react to tannin tests. For example, when ferric chloride is added to a solution of resino-tannol, iron tannate is formed as a precipitate. Other reagents used to test this relationship are potassium bichromate, lead acetate, potassium hydrate in alcoholic solution, and nitric acid. Should any of these reagents give a positive test, an exceedingly difficult problem would then confront the investigator.

Since tannin is not a single compound, but a convenient name for a related group of compounds, separate tests of the entire group would then be necessary. Moreover, as TSCHIRCH has pointed out (*loc. cit.* 1142), such a test would not necessarily prove a genetic relationship, since tannin might be merely a by-product and not its source.

Samples of the resin (ether extraction) from *B. sagittata* were submitted to the resino-tannol tests. All samples gave negative test except the one where nitric acid was used as the reagent, in which case the test was atypical. This test was so positive, however, that it served to indicate a possible relationship of another nature. Two or three drops of the pure resin were placed in concentrated nitric acid. The resin globules became dark brown, with a violent evolution of nitric oxide in the course of two minutes, accompanied by the formation of a cellulose membrane across the top of the solution. When heated, this membrane burned with a warm yellow flame and heavy smoke, leaving a black char. The odor was like that of burning celluloid. The test was then repeated with resin dissolved in 95 per cent alcohol. The reaction was delayed, not taking place for 5 minutes, but was accompanied by a more violent evolution of the gas. When the test was repeated with the resin dissolved in absolute alcohol, the test reaction did not take place for 6 minutes, and was even more violent than on either of the previous occasions. Such a reaction would indicate a relationship to cellulose or other carbohydrate.

The second group of resins are called resenes. They are the ones showing kinship to the terpenes and the fatty aldehydes. The modified cholesterol tests are applied to these substances. Two of the more common and specific ones are the Salkowsky-Hesse and Mach reactions. In the Salkowsky-Hesse test 0.002–0.003 gm. of the resin is placed in 3 cc. of chloroform and shaken with 3 cc. of concentrated sulphuric acid. The chloroform solution is then evaporated in a porcelain dish and the color of the residue noted. The color differs for various known resenes, from orange through lavender to blue, but is always a constant index for a particular resene. Substances that are not resenes do not give such color tests. In the Mach tests 0.003 gm. of the resin is placed in 1 cc.

of concentrated hydrochloric acid and evaporated in a porcelain dish and the residue washed. If the test is positive, the residue is usually blood orange or red. Both the Salkowsky-Hesse and the Mach tests were applied to the July and October resin of the *Balsamorrhiza*. The results were negative.

As previously described, the fatty oil test is made with osmic acid. A slide with a thin smear of the resin is inverted over a solution of the acid or of the crystals. The fumes of the reagent cause fatty substances to blacken. When the osmic anhydride was applied to resin of *B. sagittata*, no positive test was secured, even after prolonged application.

If resin gives an acid reaction to litmus or requires several portions of one-tenth normal sodium hydrate to neutralize, it is said to be a resinic acid. Such acids unite with ammonium hydrate and the hydrates of the alkali metals to form unstable resinic esters. A great number of these resinic acids are known, although their chemical formulae have been worked out only empirically. Certain of these acids have been distinguished by the type of ester formed with ammonium hydrate. For example, the group to which pimar acid belongs builds a very beautiful acid ammonium salt, while the group to which abietic acid belongs forms with ammonium hydrate a non-crystalline gelatinous emulsion (see TSCHIRCH, *loc. cit.* 519). The resin of *B. sagittata* gives a very decided acid test. It combines with ammonium hydrate, potassium hydrate, and sodium hydrate to form resinic esters. Moreover, the ammonium ester is an emulsoid.

The evidence gained from these tests shows that the resin of *B. sagittata* is a member of the resinic acid group, giving an ester with ammonium hydrate similar to that of abietic acid, and that it has certain relationships to carbohydrates in that it forms a nitrocellulose when reacted upon by nitric acid.

It was found that by a distillation of the resinic acid, either from the gross plant structure or from ether extracted resin, in the presence of steam, an entirely new product was formed. The substance had a tendency to crystallize upon cooling below 25°, and gave off a very characteristic pungent odor, sweetish, but very irritating to the mucous membrane. The substance was white, opaque, and

crystallized out of water in a very elaborate form, simulating frost crystals. Later it was found that it crystallized as long monoclines out of alcohol or ether. The two resene tests were applied to this substance, with positive results in both cases.

SALKOWSKY-HESSE TEST

Sulphuric acid solution after shaking: golden yellow.

Chloroform solution before evaporation: pale yellow, nearly colorless.

Residue after evaporation of chloroform in porcelain dish: first, bright yellow; later, rich dark brown; red brown; ending in deep violet.

No fluorescence.

MACH TEST

Color of residue from evaporation of alcoholic solution of resin with hydrochloric acid and ferric chloride: dark red.

This resene is saturated, failing to absorb iodine, but is weakly acid.

These positive tests, together with the general physical properties of the substance, were proof that the material under analysis was a resene, a type of fatty aldehyde. It was further discovered that all of the resinic acid was converted into resene in the process of steam distillation.

Two preparations of resene from steam distillation of spring roots were made during August 1916. One of these was placed in a glass-stoppered bottle and the other in a loosely corked vial. An examination after 6 months showed that the former preparation was in the original crystalline state, while the latter had been converted into a lemon-colored resin, and had completely lost its crystalline structure. This fact supports the view that the resene had been converted into resinic acid by an oxidative process, such as holds true for terpenes in general. This process follows the natural method expected in the plant tissues, and is the reverse of the reduction process in the presence of steam.

The discovery that resene is derived from resinic acid gave rise to the inquiry as to whether resene might not be found in the *Balsamorrhiza* plant; in short, whether there might not be a genetic connection between the two substances in the plant itself. The following methods were carried out in this inquiry: modified resene tests *en bloc* and modified Mach tests applied microchemically.

In the tests *en bloc* equal portions of *Balsamorrhiza* roots (alcoholic preservation of August material) and sprouting stem buds (fresh March material) were each placed in 5 cc. of chloroform and left for two days. The plant tissues were then removed and 5 cc. of sulphuric acid added, according to the Salkowsky-Hesse method, and the mixture thoroughly shaken. The results are given in table V.

TABLE V

	August root	March bud
Sulphuric acid solution	Pale tan	Colorless
Chloroform solution	Colorless	Colorless
Residue from evaporation	Colorless	Lavender to violet
Iridescence	None	Marked between solutions

The Mach test (modified) was used on sections of rapidly growing stem buds, just previously placed in 85 per cent alcohol. Sections of this material were cut in 95 per cent alcohol; 1 cc. of this alcohol, 1 cc. of ferric chloride, and 1 cc. of hydrochloric acid were mixed and the sections transferred to this mixture on a depression slide. The slide was then gently heated until the mixture was reduced to about 1 cc. Even from gross inspection a typical Mach test was produced in the vascular tissues. Examination under low power of the microscope showed reactions in the following places: heavy stain in the cambium and rays (identical with regions testing heavily for resin in fall tissues); specially marked test against walls of endodermis facing cortex; all through cortex and pith to more or less degree. In the heavily testing regions masses of monoclinic crystals were found, deeply impregnated with the stain from surrounding crystals that had dissolved (fig. 37). This same test was applied to roots of the August collection, preserved in 60 per cent alcohol. The results of the test were negative. This very specimen block had been used previously for resin tests and had yielded a decided resin test in the vascular and conductive areas.

These two tests, the Salkowsky-Hesse and Mach, modified to meet the needs of the material under investigation, applied to *Balsamorrhiza* material, showed a negative test for fall roots and

a uniquely strong positive test for the spring bud region. In fact, a comparison of the former test with the present one would indicate a much higher percentage of the resene in the spring bud than was necessary for a test reaction. Moreover, the Mach test both checked up the results obtained in the Salkowsky-Hesse reaction and gave the precise location of the resene in the growing bud.

A final test to check up the previous determinations consisted in placing some of the material of the fall collection and the spring collection in absolute alcohol-ether, half and half, for a period of two days, then allowing the filtered solution to evaporate. No crystals were found from a careful examination of the fall roots, yet an abundance of crystals of the monoclinic type were secured from the spring stem material.

The evidence secured from these reactions for tissues of *B. sagittata* shows (1) that resene is found in the growing plant tissues, in the meristem and conductive areas; (2) that resene is found in the same region in spring tissues where resinic acid is found in the fall tissues; and (3) that resinic acid areas in fall tissues test negatively for resene.

In the middle of May roots dug about May 1 were tested for percentage of ether extract. Such data are recorded in the ecological section of this paper. This material shows both resene and resinic acid present in tissues at this particular time of the year, when the leaves had been well developed and metabolic processes were near the zenith point.

When tests were made on various parts of the plant to discover whether a Mach test could be secured, the test was negative. These tests were made on stem and root tissue and on cotyledons and embryo within the seed coat. Later certain crystals were noted in the connective and storage tissues of the plant, spheroidal in shape, with rays arising from an eccentric umbo. The crystals were observed in material which had been preserved in alcohol *en bloc*. These crystals did not occur in fresh aqueous mounts nor in fresh material sectioned and mounted in alcohol. The type of the crystal was such and its reaction to reagents such as to establish

it as the crystallized inulin, a colloidal polysaccharide. In ordinary growing tissues these crystals are deposited in a viscous lemon-yellow mass, but in alcohol they undergo certain changes in shape. In readily permeable tissues they are laid down as granular masses, but where there is slow alcoholic penetration they are laid down as sphero-crystals. Such crystals are well illustrated and their location shown in fig. 36 (*si*). They are found in connective tissue, especially in the rays and in the inner cortex. In this same specimen the canals are filled with resin. The semiviscous, semigranular resene is well brought out in fig. 16, the section of a very young subsidiary root without secondary thickenings yet developed. In fig. 17, the section of a subsidiary root further developed, is shown in the more permeable outer region of the cortex the semiviscous, semigranular inulin, while the sphero-crystals are found in the inner cortex, not so permeable to alcohol.

Other observations on the growing stem buds showed the following relationships. Young etiolated stem buds showed no inulin, while green stem buds were filled with inulin. Such observations are proof that the result of the photosynthetic process in *B. sagittata* is inulin. Such a substitute for starch is found in the related Compositae, *Helianthus annuus*, *Inula Helenium*, and for roots of *Dahlia* spp.

As the microchemical tests progressed, evidence became stronger that a genetic relationship existed progressively in turn between each two of the three products found in *Balsamorrhiza*, namely, inulin, resene, and resinic acid. The hypothesis built up on this evidence may be stated thus:

Inulin < resene < resinic acid; in other words, inulin, a polysaccharide, formed in the plant in the process of photosynthesis, by a process of polymerization is changed to resene, and by reduction the resene is altered to resinic acid, a waste product of the plant. The direct evidence supporting this view may well be summarized at this point: (1) etiolated stem buds contain neither inulin nor resene, while green leaves test for both coincidentally; (2) resene and resinic acid are found in the stem and root at the same time; resene more frequently occurs in conductive tissue and

resinic acid in ducts and canals; (3) resene is derived from resinic acid in the presence of steam; (4) resene is converted into resinic acid in the presence of oxygen.

A suggestion of the effects of resinic ester and resene on the vegetative growth of *B. sagittata*, and in consequence an idea of the physiological nature of the products, is shown by the effect of these substances on the living protoplasm. Although this plant is not listed among the poisonous plants of the western stock ranges along with the death camas (*Zygadenus venenosus*), the loco weeds (*Aragallus* and *Astragalus* spp.), the larkspurs and the lupine (*Lupinus ornatus*), it is the common belief of stockmen that the root and stem of *B. sagittata* often cause stock poisoning, especially among sheep. Certain experimental proof of this toxic property of the resinic esters and the resene of this plant will be presented.

A neutral potassium resinate was prepared from titration with a saturated solution of potassium hydrate and an alcoholic solution of the resinic acid. The alcohol was allowed to evaporate and the ester dissolved in water to make a saturated solution. A few drops of this solution were introduced into a watchglass containing filaments of *Chara* in 10 cc. of tap water. Such a dilute solution of the ester was not sufficient to effect any osmotic changes in any appreciable way. Observations were made in the following manner.

A filament of *Chara* had previously been singled out and the rate of flow of the protoplasm under low power of the microscope noted. A convenient distance was chosen on a blank paper at the side of the microscope, and by means of a camera attachment the time for this distance flow was then taken to ascertain the average time flow. Such an average in this case was found to be 20 seconds, with a maximum at 22 and a minimum at 18 seconds. A final normal reading was taken at 9:14 A.M. and the specified amount of the ester introduced. Five minutes later the time flow was 30 seconds; 6 minutes later, 27 seconds; 7 minutes later, 25 seconds; 8 minutes later, 23 seconds; 13 minutes later, 23 seconds; 15 minutes later, 20 seconds; and at 10:38 A.M., 19 seconds. This shows an immediate effect in the time flow and a rather rapid recovery. When a double dose of the solution, that is, 6 drops of the

saturated solution to 10 cc. of water, was used, the following data were secured:

12:40–12:58 P.M.	Time flow 20 seconds
12:59 ester introduced	
1:00	25
1:01	30
1:02	30
2:03	35
2:04	35
4:18	40
4:22	30
4:25	32
4:26	33
4:30	30
5:00	60
5:04	60
9:00 A.M. following, death of the filament, but with no plasmolysis	

Check experiments on a new filament with the same toxic doses were used, with similar results.

TRUE (**20**), working on *Lupinus albus*, found for inorganic acids that the H ion produced the greater toxic effect on the vitality than the Na ion of the sodium salt. The same was correspondingly true for organic acids, with the toxicity proportional to the dissociation of the H ion. It may be noted in passing that the resinic acid would probably be more toxic than the potassium salt.

In the process of steam distillation of the resene condensation at 10° C. is not complete. Unless the apparatus is inclosed in a hood with a good vent, the room soon becomes permeated with the volatile resene. It has a characteristically sweetish odor, very terebinthine in nature. During a period of 3–4 hours the writer was in the immediate vicinity of a resene still, with the room temperature at 22° C. and the condenser at 10°. Certain pains developed under the eyes with sharp, shooting pains in the occipital region. Also a dull pain developed in the spinal cord, mostly in the lumbar region. Within a short time a high fever arose (102–104° F.), alternating with chills. At times, as the chills abated, the blood coursed through the head, seemingly laden with fire. A tickling sensation

was produced in the respiratory tract, centering in the bronchi, giving rise to coughing which seemed almost to split the head. This condition continued for almost 36 hours, at the end of which time the fever began to abate and the acute pains to leave. The irritation of the lungs and bronchi continued for more than a week before it was relieved.

A similar test was made, except that the windows were wide open and a strong breeze blew the vapors away from the writer. No ill effects were observed. Again, when the apparatus was thoroughly hooded, no harmful effects were felt.

In steam distillation, where water and resene distil and condense simultaneously, the resene collects on top of the distilled water. It was found, however, that a fine grade of filter paper does not free the filtrate of the resene product, evidently due to a colloidal suspension of the resene in the water. When exposed to the air and allowed to evaporate for some time, some of the suspensoid is precipitated in crystal form. The toxic effect of this colloidal suspensoid of resene is brought out by the effect on the protoplasmic flow of *Chara*.

Resene was distilled from fall roots of *B. sagittata* and allowed to condense at 10° C. along with water. This water was filtered and the filtrate allowed to act on *Chara* sp. It tested tannin free. The normal time flow for a unit distance was found to be 10, 10, 10, 10, 10, 10, etc. The *Chara* was then transferred to this colloidal suspension, in parts one of the suspension to nine of water free from the suspensoid. Observations on the effect of the resene on the protoplasmic flow were made for 90 minutes. The observations are recorded in table VI.

A study of the data shows a marked lowering of the vitality immediately upon the introduction of the resene, followed by increased activity of the protoplasm. The lowering of the protoplasmic flow occurred almost rhythmically, followed by an alternate rhythmic increase in vitality. This continued until the final decrease in flow, with death ensuing. The amount introduced was much less than that required for an appreciable exosmosis (text fig. 2).

The writer recognizes that a correct quantitative measurement of the toxicity of resene and resinic esters is desirable, and has under

way such a test, together with a physiological standardization of the products.

TABLE VI

RECORD OF EFFECT OF COLLOIDAL SOLUTION OF RESENE FROM *B. sagittata* ON PROTOPLASMIC FLOW OF *Chara* SP. THROUGH UNIT DISTANCE OF 0.465 MM.

Time	Time flow	Time	Time flow	Time	Time flow
7:15 P.M.	10	7:41 P.M.	18	8:09 P.M.	12
7:16	10	7:42	No record	8:10	13
7:17	10	7:43	13	8:11	14
7:18	10	7:44	12	8:12	15
7:19	10	7:45	8	8:13	14
7:20*		7:46	8	8:14	No record
7:21*		7:47	10	8:15	"
7:22*		7:48	10	8:16	13
7:23	15	7:49	12	8:17	13
7:24	15	7:50	12	8:18	13
7:25	16	7:51	14	8:19	19
7:26	14	7:52	18	8:20	20
7:27		7:53	13	8:21	15
7:28	10	7:54	18	8:22	14
7:29	8	7:55	18	8:23	14
7:30	6	7:56	22	8:24	No record
7:31	12	7:57	23	8:25	"
7:32	15	7:58	28	8:27	18
7:33	12	7:59	22	8:28	No record
7:34	10	8:00	18	8:29	14
7:34.5	8	8:01	13	8:30	11
7:35	15	8:02	12	8:31	14
7:35.5	20	8:03	12	8:32	12
7:36	17	8:04	12	8.33	15
7:37	16	8:05	14	8:34	24
7:38	14	8:06	11	8:35	∞ with death
7:39	14	8:07	No record		
7:40	18	8:08	16		

*Resene introduced here.

Discussion

A survey of the study of resin secretion makes it evident that the problem can be considered logically only in the light of the threefold evidence, the ecological, the anatomical, and the physiological. The assertion of TSCHIRCH (*loc. cit.* p. 1145) that "we shall assuredly not arrive at a conclusion by anatomico-microchemical" investigations is only a half truth. All phases of the problem must be carefully weighed in order to understand the problem. In this evidence the anatomico-microchemical data surely have their place.

The writer does not claim that the particular solution of resin secretion for *B. sagittata* is a complete solution for resin secretion

in all plants. However, it suggests a method of attack to be followed in working out other problems of a similar nature and scope.

The problem of resin secretion in *B. sagittata* is one limited to the field of an acid resin, non-tannin testing. For this type of resin, perhaps by far the most common, theories have been advanced

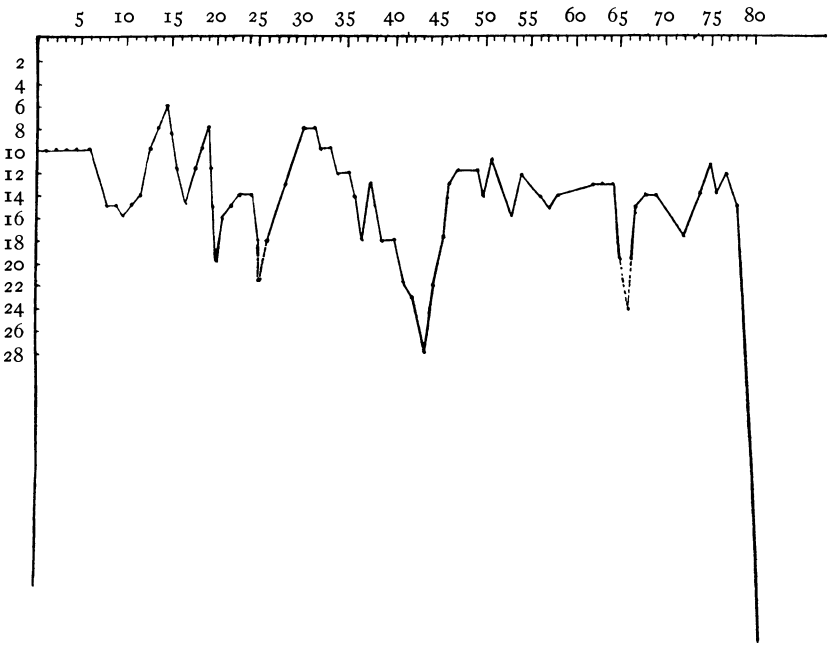

FIG. 2.—Coordinate plot, representing effect of balsamoresene on protoplasmic activity of *Chara* sp; the ordinates represent time flow and abscissas sequence of time.

advocating the origin from carbohydrates on the one hand, and the origin from terpenes on the other. Foremost of those advocating the former theory was WIESNER (**24**). He assumed that resins are derived from carbohydrates, specifically starch, by polymerization and reduction. As he knew, this fails to account for resin in the pine family, where there is a maximum production of resin but very little starch formation. WIESNER explained this on the basis that gallo-tannic acids operated to produce the change in this family.

Recently FRANKFORTER (5), working from the biochemical angle, has criticized WIESNER'S theory on the ground that it is unreasonable to suppose a complex starch molecule would be formed and then broken down into the terpene and resene radical. The criticism is not well founded, because it fails to consider the fundamental function of resin secretion. In other words, this criticism evades the anatomico-physiological viewpoint which TSCHIRCH claimed could never solve the problem; but in evading this point of view the entire meaning of resin secretion is overlooked.

The theory of resin formation from terpenes is supported both by theoretical and actual evidence. Such a theory was postulated by BAEYER (1), who obtained several resins by oxidation of fatty aldehydes, although these resins were unlike any found in nature.

The evidence presented from a biochemical study of *B. sagittata* shows (1) the presence of a resinic acid, with reaction similar to that for abietic acid; (2) production of the fatty aldehyde, *balsamoresene*, by steam distillation of the resinic acid and the formation of the resinic acid from resene in the presence of oxygen; and (3) a strong test for inulin, a polysaccharide, in photosynthetic and conductive areas, in conjunction with balsamoresene. This evidence, added to the comparative studies, warrants the assumption that *balsamoresinic acid is derived from balsamoresene*, which, in turn, is derived from inulin by polymerization.

Although the evidence already presented in the physiological section is the most convincing, yet that secured from the ecological data and anatomical observations brings the problem to a clearer focus. HABERLANDT (*loc. cit.* p. 525), referring to the coincident bundle traces and secretory canals, concludes that "the reason for the frequent association of secretory passages with leptome strands and other vascular tissues is, therefore, in all probability, an ecological one." Furthermore, "the substances contained in these passages are often of such a kind as to be capable of affording 'chemical protection' against noxious animals; hence small assailants which have penetrated into the interior of an organ will be more or less effectually discouraged from attacking the conductive strands, the continuity of which is so vital to the well-being of the

plant, if the latter are protected by a series of secretory ducts (or excretory sacs)."

A criticism of this theory lies in the fact that it fails to comprehend the origin of resin per se, and tries to explain its reason for existence *ab exteriore*. The ecological evidence from *B. sagittata* would discourage any such reason for resin secretion. As has been described, diptera, acarinids, and nematodes parasitize the growing and reproductive parts of the plant. The fly nymph lays the eggs between the parts of the flower head, where the grub hatches and worms its way through the tissue, irrespective of resin canals, effectually limiting the source of nourishment of the ripening seeds and causing a high percentage of non-viability. The mites suck the juices of the conductive tissue, especially at the bases of the new stems and petioles, where there is an abundance of resin in the tissues. The nematodes bore into the conductive tissues of the leaf and bud and cause a withering and decay right in the resin secreting areas.

Another ecological reason assigned for resin secretion is its protection against mechanical injury to cortex. It is based on the ground that such injured places are often covered by a resin covering. Undoubtedly this is often true, but it must be considered a secondary function, not at all the fundamental reason for resin secretion.

The fundamental underlying reason for resin secretion lies in the essential toxic nature of the resin to the plant itself. The resin is a by-product, formed in the metabolic activities of the plant. It is harmful to the plant, as judged from its effects on other organic tissues and from the storage of the product within special tubes or canals in the endodermal region, near to the place of greatest activity and growth. Since these resene and resinic acid products are toxic, they may be used as a guard against mechanical and parasitic injury. They may or may not be effective in such capacity.

Moreover, the anatomical observations verify this hypothesis. The balsamoresinic acid and the balsamoresene develop in cambium and other meristematic regions. They are carried outward by the rays and phloem strands until they reach the resin canals, where

they are laid down. Such seems to be the significance of the penetration of the phloem strands into the cortical areas through the Casparian strip (fig. 13).

Much emphasis has been placed by TSCHIRCH (*loc. cit.* p. 1118) on the non-permeability of resinic acid through cell walls. This author contends that resin is laid down where it is formed. Yet in an alkaline medium, such as is frequently if not always found in growing tissues, an unstable resin ester would be formed, undoubtedly capable of penetrating cell walls. Then, too, the fact remains that a considerable part of the resin forms in the meristem and is transferred to the canals, else it would never get to the canals. Such a transfer could be accomplished in the form of a temporary ester. On the other hand, the microchemical observations show that the larger amount of the product is transferred as balsamoresene and changed to the acid in the vicinity of the cells immediately surrounding the canals or in the canals themselves. There is even evidence to support the view that inulin may be changed into resene and later into resinic acid in the vicinity of the canals. In fact, such a change is actually shown in progress in fig. 36. There seems to be no specific way for the translocation of the by-products to the resin ducts. It may be accomplished by a temporary ester formation, or by the translocation in the form of balsamoresene, and later changed into resinic acid, and it may be centrifugally distributed as a fractional depolymer of inulin, and consequently changed to resene and resinic acid near the canals. Any one of these means would satisfy the needs of a translocation in a dialyzable form.

Summary

1. *Balsamorrhiza sagittata* is the dominant member of its habitat in the inter-mountain region. The plant depends largely upon growth of the rootstock for propagation. It does not produce flowers until the third or fourth season. A hardy rootstock accounts for its dominance, since the viability of the seeds is small, due to parasitic infection.

2. The radicle has the tetrarch type of development. The resin canals of the root arise in two concentric rows above and

including the hardy mid-rootstock, with radial canals between the longitudinal canals of the two series. Only the outer of the two series of canals is found in the lowest portion of the rootstock and the subsidiary roots. A twofold series of canals is found in the stem and leaves, an outer series in the sinuses of the cortex opposite the interfascicular regions, and a second inner series in the pith opposite the hadrome elements. The root canals and the stem canals arise as two separate systems and remain distinct. The resin canals do not arise until long after resin is formed in the meristem.

3. *Balsamoresene* and *balsamoresinic* acid are formed in *B. sagittata* from *inulin*, probably by polymerization and reduction. The resene and resinic acid are essentially toxic in nature. The resene is the immediate substance from which resinic acid is formed. The secretory process is dependent on physiological activity in the meristem of the plant, in which inulin is used in anabolism and resene and resinic acid are derived as waste products in the plant. The resinic acid and resene are transferred to the secretory canals, where they are stored.

4. To summarize, the study of *B. sagittata*, with especial emphasis to the meaning of resin secretion, has developed certain facts regarding the purpose of resin secretion. In the growth of the plant a polysaccharide, inulin, produced during photosynthesis, is broken down, causing a by-product, balsamoresene, to be produced. This resene is changed to resinic acid. On account of the probable toxic nature of the resene and resinic acid to the plant, they are translocated to schizogenously formed ducts of endodermal origin, where they are stored as resinic acid.

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LITERATURE CITED

1. BAEYER, ADOLPH, Über Condensation und Polymerie. Ann. Chem. Suppl. **5**:79–95. 1867.
2. CALVERT, AGNES, and BOODLE, L. A., On laticiferous tissue in the pith of *Manihot Glaziovii* and on the presence of nuclei in the tissues. Ann. Botany **1**:55–62. *pl. 1.* 1887.

3. COL, M. A., Recherches sur l'appareil sécréteur interne des Composées. Jour. Botanique **17**:252–318; **18**:110–134. 1903–4.
4. DEBARY, A., Comparative anatomy of phanerogams and ferns. 1884.
5. FRANKFORTER, GEORGE B., The resins and their chemical relations to the terpenes. Merck's Rept. 1913:209–211.
6. HABERLANDT, G. Physiological Plant Anatomy. 1914.
7. HOLM, THEO., Medicinal plants of North America. 77. *Solidago odora* Ait. Merck's Rept. 1913, pp. 252–254.
8. KARSTEN, H., Über die Entstehung des Harzes, Wachses, Gummis, und Schleims durch die Assimilierende Thätigkeit der Zellmembran. Bot. Zeit. **15**:313–321. 1857.
9. KIRKWOOD, J. E., The propagation of guayule by seed. Ann. Rev. Trop. Agric. **1**:34–43; 77–84. 1910.
10. LEE, E., Observations on the seedling anatomy of certain Sympetalae. II. Compositae. Ann. Botany **28**:303–329. *figs.* 2. 1914.
11. LLOYD, FRANCIS E., Guayule (*Parthenium argentatum* Gray), a rubber plant of the Chihuahuan Desert. Carnegie Inst. Wash. pp. 213. *pls.* 46. 1911.
12. MAYR, H., Entstehung und Verteilung der Sekretions-Organe der Fichte und Lärche. Eine vergleichend-anatomische Studie. Bot. Centralbl. **20**: 23–31. 1884.
13. MEYEN, F. J. F., Über der Sekretions-Organe der Pflanzen. Berlin. pp. 18. 1837. Quoted from TSCHIRCH.
14. ———, Neues System der Pflanzen-Physiologie. II. 1839.
15. MUELLER, N. J. C., Untersuchungen über die Verteilung der Harze, aetherischen Oelen, Gummi, und Gummiharzen, und die Stellung der Sekretions behaelter im Pflanzen-körper. Jahrb. Wiss. Bot. **5**:387–421. *pls.* 47–53. 1866.
16. NUTTALL, THOS., Description of new species and genera of plants in the natural order of the Compositae. *Balsamorrhiza sagittata.* Trans. Amer. Phil. Soc. **7**:350. 1841.
17. RUSSOW, E., Zur Kenntnis des Holzes, in sonderheit des Coniferenholzes. Bot. Centralbl. **4**: *pls.* 5. 1883.
18. SANIO, CARL, Einige Bemerkungen über den Bau des Holzes: V. Über Gerbstoff im Holze nebst einigen Bemerkungen über Gerbstoff überhaupt. Bot. Zeit. **18**:213, 214. 1860.
19. SAYRE, L. E., and KELLEY, HERMA T., A new drug, *Balsamorrhiza terebinthacea.* Drug. Circ. Feb. 32, 33. 1897.
20. TRUE, R. H., The toxic action of a series of acids and of their sodium salts on *Lupinus albus.* Amer. Jour. Sci. IV. **9**:183–192. 1900.
21. TSCHIRCH, A., Die Harz und die Harz-behaelter. pp. 1268. Leipzig. 1906.
22. VAN TIEGHEM, PH., Les canaux sécréteurs des plantes. Ann. Sci. Nat. Bot. V. **16**:96–147. 1872.

23. VILLUEMIN, M., Remarques sur la situation de l'appareil sécréteur des Composées. Bull. Soc. Bot. France **31**:112–116. 1884.
24. WIESNER, J., Über der Entstehung des Harzes im Innern der Pflanze. Sitz. Ber. Akad. Wiss.; rev. in Chem. Centralbl. **36**:756–763. 1865.
25. WHITTELSEY, GEORGE, Guayule rubber. I and II. Jour. Ind. and Eng. Chem. **1**: no. 4. 1909.
26. WIGAND, A., Über die Deorganization der Pflanzenzellen inbesondere über die physiologische Bedeutung von Gummi und Harz. Jahrb. Wiss. Bot. **3**:115–182. *pls. 3.* 1863.

EXPLANATION OF PLATES XXVIII–XXXI

FIGS. 1–6.—Successive stages in germination of seeds of *Balsamorrhiza sagittata:* fig. 1, seed coat bursting, with hypocotyl protruding, 15 days' growth; figs. 2–5, stages from 20 to 40 days' growth; fig. 6, stage showing 60 days' growth; note type of venation in plumule; figs. 1–5, ×1.5; fig. 6, ×1.

FIGS. 7–10.—Successive stages in development of bundle anatomy of seedlings: fig. 7, formation of protoxylem, other tissues yet undifferentiated; fig. 8, protoxylem (*px*) well defined, rapid differentiation of procambium (*pc*) in region where protophloem originates; fig. 9, rapid division of cambium to form secondary xylem elements (*mx*) and phloem (*ph*); fig. 10, suberization of endodermis (*en*) beginning, secondary xylem, the metaxylem, well differentiated; ×112.

FIG. 11.—Gradation of tracheids from spiral protoxylem to true eyelet type of metaxylem; taken in region of fig. 10; ×150.

FIG. 12.—Detail of suberized walls in region of endodermis, defining "H" type of thickening: *ex*, toward cortex; *in*, toward leptome.

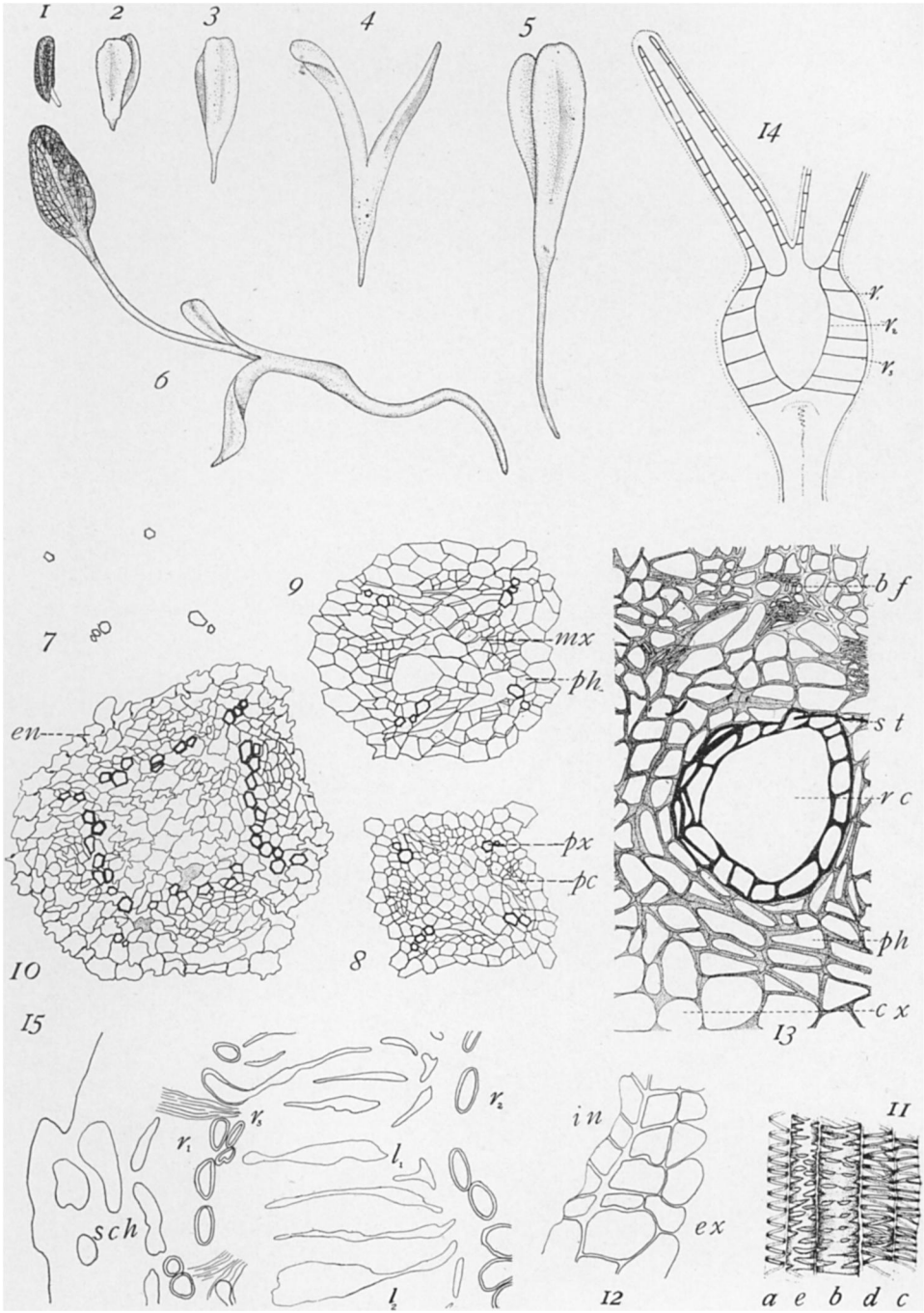
FIG. 13.—Section through 10 mm. root, illustrating passage of phloem strands through endodermis into cortex in region of canals: *ph*, phloem cells; *rc*, resin canals; *bf*, strands of bast fibers; *cx*, cells of cortex; *st*, suberized thickening of endodermis; ×150.

FIG. 14.—Diagram of resin canals in longitudinal section through stout rootstock and root branches: r_1, outer series of canals; r_2, inner series of canals; r_3, anastomosing radial connections; note inner series ends in stele just above tap root; ×0.75.

FIG. 15.—Transverse section through old root: r_1, outer series of canals; r_2, inner series of canals; r_3, radial connections; *sch*, sclerome groups; l_1, l_2, lysigenous splittings of rays; ×150.

FIG. 16.—Section through young subsidiary root with stele yet unsclerified, showing bundle anatomy: *px*, protoxylem; *cx*, cortex; *hyp*, hypodermis; *e*, epidermis; note deposits of inulin in granular masses; type of root readily permeable to alcohol; ×150.

FIG. 17.—Section through subsidiary root with moderate thickenings; note suberization of endodermis, rapid division of cambium, metaxylem ele-



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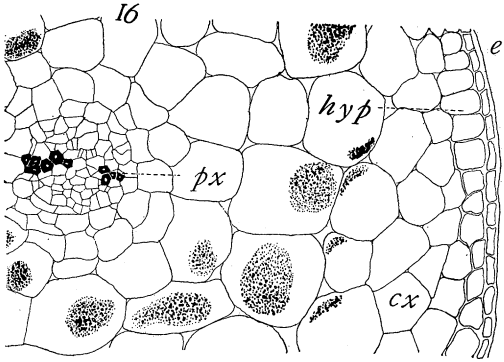

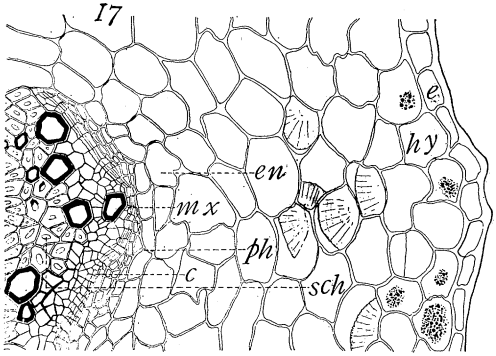

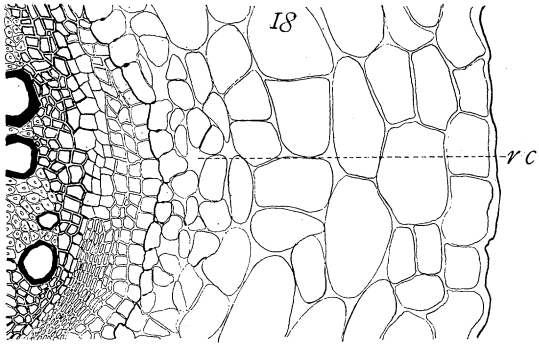

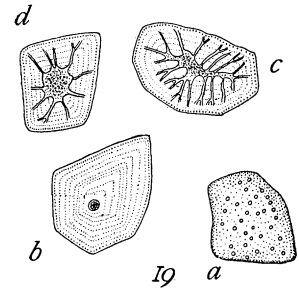

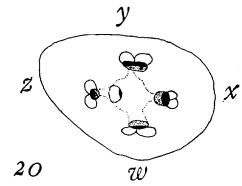

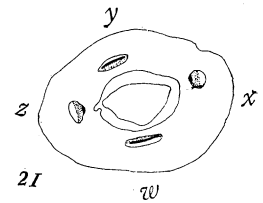

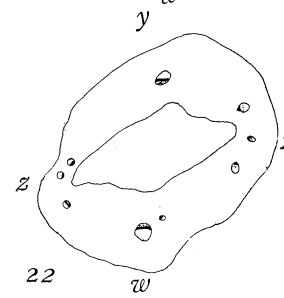

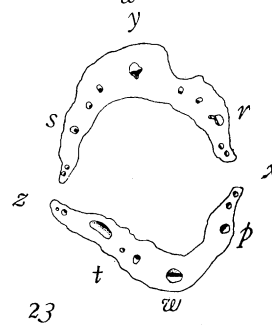

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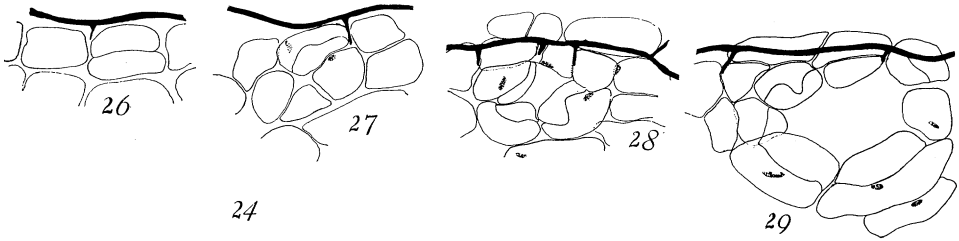

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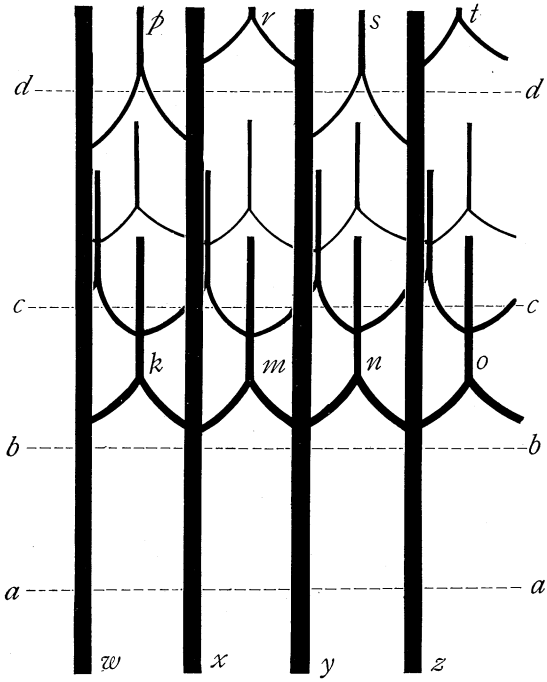

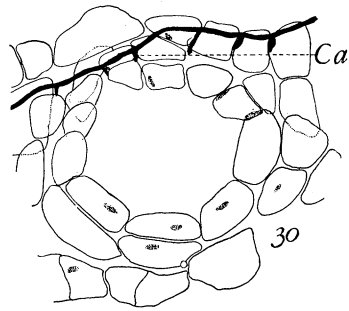

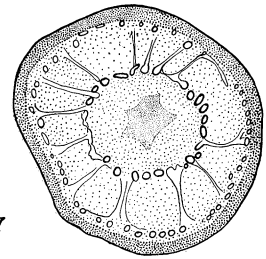

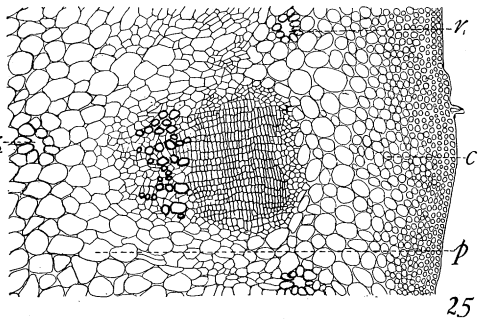

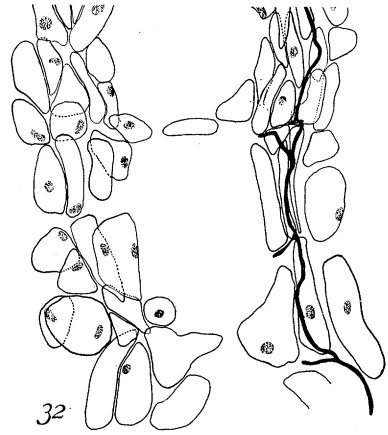

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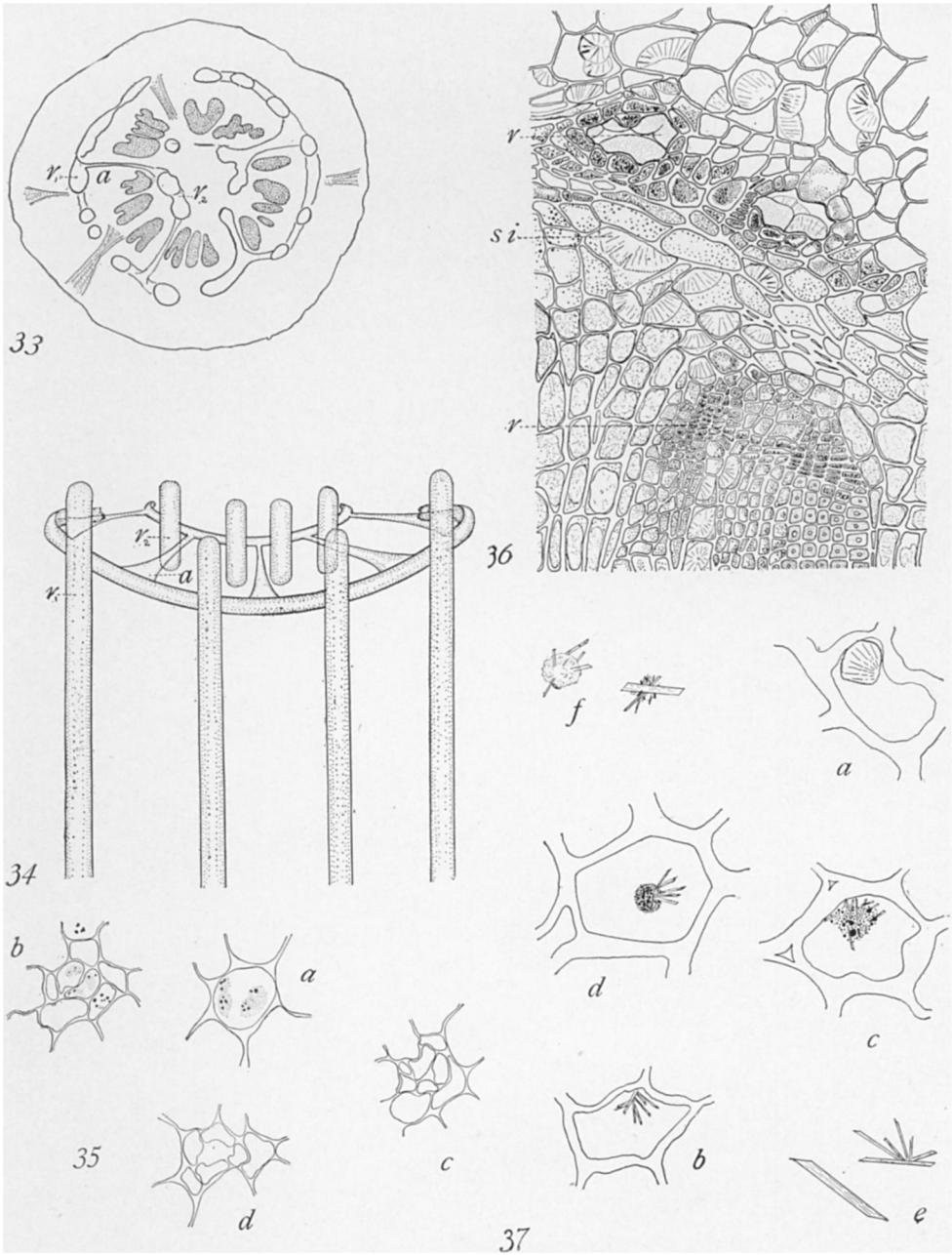

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ments, and sclerified stele; inulin deposits in outer cortex granular; in inner cortex, sphero-crystals; *mx*, metaxylem; *ph*, phloem; *c*, cambium; *sch*, sclerome; *en*, endodermis; *hy*, hypodermis; *e*, epidermis; ×150.

FIG. 18.—Section through old subsidiary root, showing formation of resin canals in region of endodermis: *rc*, resin canals; ×150.

FIG. 19.—Detail of sections of stone cells: *a*, section at edge of cell showing unsclerified pores; *b*, border of internal opening; *c*, *d*, through center of cells; *a*, *b*, *d*, longitudinal sections; *c*, transverse sections; ×300.

FIGS. 20–23.—Sections through critical levels of a 2 mm. seedling: fig. 20, region of hypocotyl; fig. 21, lower cotyledonary collar; fig. 22, upper cotyledonary collar; fig. 23, lower reaches of cotyledons; *w*, *x*, *y*, *z*, primary bundle traces in hypocotyl; *p*, *r*, *s*, *t*, secondary bundles of cotyledonary collar; white areas in bundles, phloem; black areas, xylem; dotted areas, metaxylem; ×60.

FIG. 24.—Longitudinal diagram of bundle traces in region of hypocotyl and lower epicotyl, reduced to one plane: *k*, *m*, *n*, *o*, primary traces of epicotyl; other designations as in figs. 20–23.

FIG. 25.—Transverse section through peduncle: *p*, pith; *c*, cortex; r_1, resin canals of outer series; r_2, resin canals of inner series; ×150.

FIGS. 26–30.—Cross-sections of resin canals of root at various stages of development: fig. 26, first periclinal division of initial endodermal cell; fig. 27, first oblique division; figs. 28, 29, progressive stages in formation; fig. 30, fully developed canal; *Ca*, Casparian thickening; ×150.

FIG. 31.—Transverse section of old root (4 or 5 years), showing two series of canals and radial anastomoses; ×1.

FIG. 32.—Longitudinal radial section of fully developed canal; ×150.

FIG. 33.—Transverse section of young seedling in hypocotyledonary region, showing radial anastomoses of two series of resin canals in root system: r_1, outer series; r_2, inner series; *a*, radial canals.

FIG. 34.—Schematic diagram from sections, illustrating extent and connections of resin canals of 5 mm. seedling, in region of hypocotyl: r_1, outer series of canals; r_2, inner series of canals; *a*, radial canals.

FIG. 35.—Section through origin of resin canals of stem: *a*, initial canal cell in process of division; *b*, cells dividing a second time; *c*, *d*, subsequent divisions to form canals; ×300.

FIG. 36.—Section of 3-year-old root, stained to show distribution of inulin and resin: *si*, sphero-crystals of inulin; *r*, *r*, resin deposits.

FIG. 37.—Detailed sketch of inulin, resene, and resinic acid: *a–d*, within pith cell of stem; *e*, *f*, deposited from evaporation of alcoholic solution; *a*, crystal of inulin; *b*, crystals of resene within cell; *c*, *d*, resene crystals imbedded in resin masses; *e*, detail of resene crystal; *f*, resene crystal imbedded in resin masses; ×150.